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REPORTS

Cyclic GMP System in Epidermis: II. Histamine Stimulates Cyclic GMP Formation

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In search of hormones and drugs which might elevate the intracellular level of cyclic GMP in pig epidermis, we have tested the effects of carbachol, concanavalin A, prostaglandin $F_{2\alpha}$, insulin, epinephrine, Vitamin A acid and histamine. Only histamine was found to cause consistent activation of the cyclic GMP system. Epidermal slices incubated in Hank's balanced salt solution with histamine exhibited 2- to 4-fold increases in intracellular cyclic GMP concentrations. Histamine's effect on cyclic GMP was not due to contamination by the 1000-fold higher level of cyclic AMP. The time course after histamine addition showed maximal response for cyclic GMP at 1 min and for cyclic AMP at 5 min. Leakage of cyclic GMP into the media was much more marked than that of cyclic AMP. Maximal histamine activation of the cyclic GMP system required a 1 mM concentration, whereas that for the cyclic AMP system required only a 100 μM final concentration. 3-Isobutyl, 1-methylxanthine (IBMX) highly potentiated the effect of histamine on both cyclic nucleotides, but the synergistic effect was more marked in relation to cyclic GMP accumulation. Histamine activation of the cyclic AMP system was specifically inhibited by an (H_2) inhibitor. Although both (H_1) and (H_2) inhibitors significantly reduced histamine activation of the cyclic GMP system, the inhibition by the (H_1) inhibitor was predominant. These results indicate that the pig epidermis has a weak but distinct histamine-guanylate cyclase system.

Recent reports indicate that cyclic GMP in mammalial tissue may play an important biological role in cell growth, and malignant transformation [1–8]. In many cases an increase in cyclic GMP is implicated in rapid growth [1–6], while in others a low cyclic GMP level is implicated in cell proliferation [9,10]. The effects of cyclic GMP are so variable and diverse depending on the kinds and conditions of organs involved that any generalization of their role in growth and differentiation is extremely difficult (for a recent review; see reference 7). At present the only way to study its role appears to be to select a specific organ as the subject and to define the experimental conditions rigidly.

Information on the cyclic GMP system in epidermis has been extremely limited. One study reported that in psoriasis the level of cyclic GMP was increased [8]; but another study showed that the addition of cyclic GMP or dituyryl cyclic GMP did not significantly influence the rate of the epidermal mitosis in culture [11].

We now report the effects on skin of hormones and chemicals which have been reported to stimulate the guanylate cyclase in other tissues. Only histamine has modest stimulatory effect on the epidermal guanylate cyclase system of pig skin.

MATERIALS AND METHODS

Thin slices of epidermis were obtained from domestic pigs weighing 6–8 kg by a Castroviejo keratome set at 0.3 mm depth. The epidermal slices were cut into 5 × 5 mm squares and kept at 4°C in Hank's balanced salt solution. After preincubation of the skin squares at 37°C for 15–20 min to stabilize both cyclic-AMP and cyclic-GMP levels in the tissue, 4 squares each were incubated with test hormones or drugs for short periods as specified below. Cyclic nucleotides were extracted and measured as described in the preceding paper [12]. However the succinylation step before the radioimmunoassay was replaced by acetylation, which yielded consistent results for measurements of both cyclic AMP and cyclic GMP [13,14]. Protein was measured by the method of Lowry et al [15] after dissolving the perchloric acid-precipitate in 1 N NaOH at room temperature for 1–2 hr. Sources for radioactive and nonradioactive drugs and chemicals are the same as described in the preceding paper [12].

Epinephrine was the product of Parke Davis (Detroit, Mich.), and metiamide was a kind gift from Dr. Brimblecombe, Smith, Kline and French Lab., U.K. Chemicals and drugs were prepared fresh and the pH of the solution was adjusted to 7 before each experiment.

RESULTS

The intracellular concentrations of cyclic GMP and cyclic AMP rose in response to histamine (Fig 1). The intracellular cyclic GMP level reached maximal accumulation at 1 min and remained relatively high for 5 min. In contrast, the level of cyclic AMP continuously increased and reached a peak in 5 min. Histamine caused 2- to 4-fold accumulation of cyclic GMP as compared with a 20-fold accumulation of cyclic AMP. Cyclic GMP accumulated in the media, while cyclic AMP did not. Cyclic GMP appears to "leak" from epidermal cells more easily than cyclic AMP. Cyclic GMP accumulation in the skin was dose dependent and the maximal effect was observed at 1 mM (Fig 2), whereas in previous studies cyclic AMP accumulation was nearly maximal at 100 μM (c.f. reference 16).

Histamine and a potent cyclic nucleotide-phosphodiesterase inhibitor, 3-isobutyl, 1-methylxanthine (IBMX), were added singly or in combination (Fig 3). IBMX by itself greatly enhanced the cyclic GMP accumulation, but elevated only slightly the cyclic AMP level. Histamine alone caused much higher stimulation of cyclic AMP accumulation. Simultaneous addition of both IBMX and histamine was synergistic for both cyclic GMP and cyclic AMP, but the degree of synergism was much higher for cyclic GMP than cyclic AMP.

Diphenhydramine, a histamine (H_1) antagonist, inhibited

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Abbreviations:
IBMX: 3-isobutyl, 1-methylxanthine

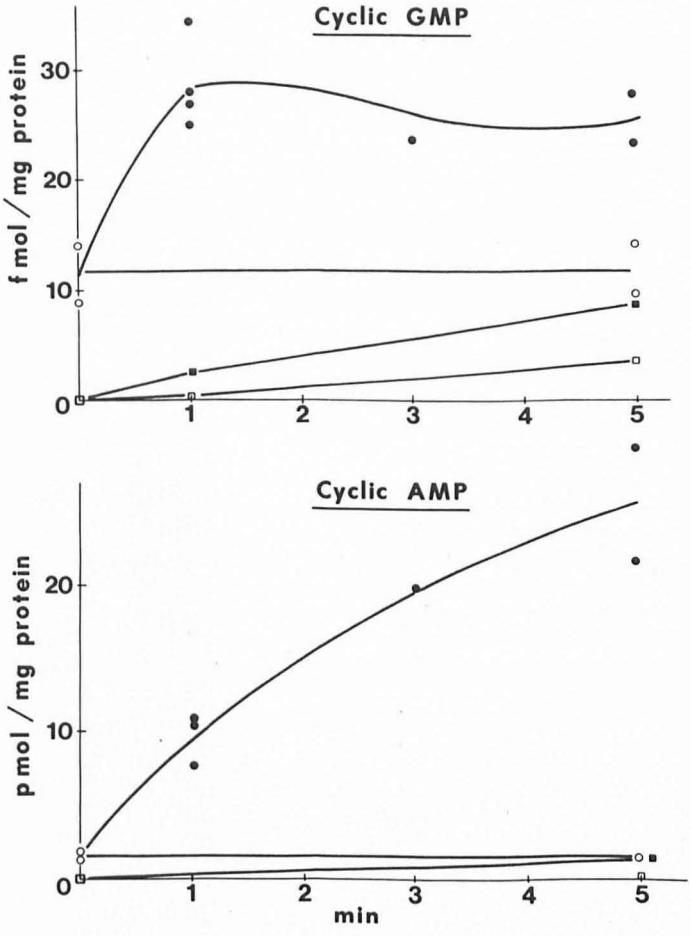

FIG 1. Time course of the effect of histamine. After preincubation for 15 min, the epidermal squares were incubated for the indicated periods in Hank's balanced salt solution containing 1 mM histamine. No phosphodiesterase inhibitor was added to the medium. Each value is an average of 2 determinations unless otherwise specified. (○)---intracellular levels of cyclic nucleotides in the control epidermis (no histamine added) and (●)---those in the media with histamine. The cyclic nucleotide values in the media, with histamine (■) and without histamine, i.e. "control; (□), were expressed on a per mg protein basis of the epidermal squares in the particular incubation.

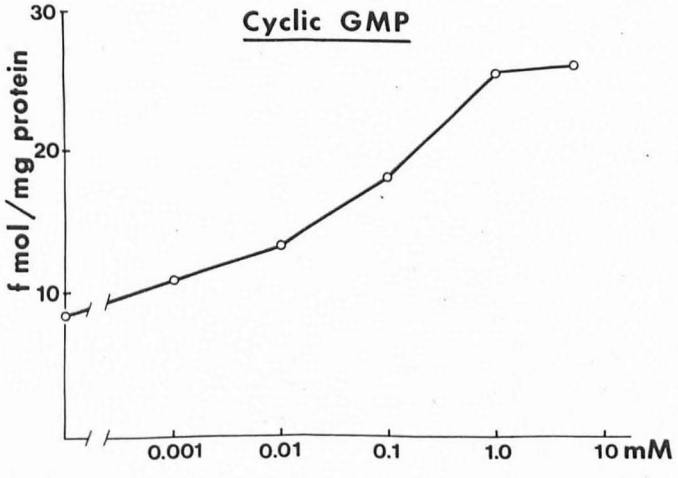

FIG 2. The effect of histamine concentration. The epidermis were incubated with various concentrations of histamine for 1 min. Preincubation was done for 15 min. No phosphodiesterase inhibitor was added to the medium. (An average of 2 experiments, each assayed in duplicate.)

histamine stimulation of the cyclic GMP system, but did not inhibit that of the cyclic AMP system (Fig 4). The effect of another histamine (H_1) inhibitor, pyrilamine was also tested with essentially the same results. Pyrilamine at a final concentration of 100 μM and 1 mM inhibited 90% of the cyclic GMP increase due to histamine (1 mM) activation. No effect of pyrilamine on histamine stimulation of the cyclic AMP system was observed. Metiamide, a histamine (H_2) inhibitor, at 1 mM final concentration which was equal to the histamine concentration, inhibited 95% of the cyclic AMP activation, but demonstrated only 60% inhibition of the cyclic GMP activation (Fig 5). At 100 μM (1/10 of the histamine concentration), it inhibited nearly 70% of the cyclic AMP accumulation and only 40% of the cyclic GMP accumulation.

To ensure that the cyclic GMP fraction we have been assaying does not include spill-over from the cyclic AMP fraction, we examined the effects of epinephrine on the epidermal aden-

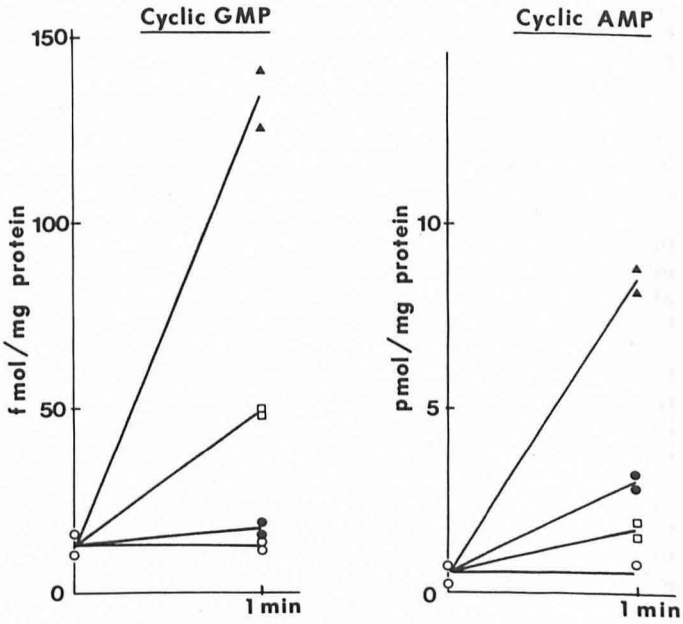

FIG 3. Histamine with or without a phosphodiesterase inhibitor (IBMX). After preincubation, the epidermal squares were incubated for 1 min in Hank's balanced salt solution, containing 100 μM IBMX (□), 100 μM histamine (●) or 100 μM each of both drugs (▲). The control medium (○) contained no drugs. (An average of 2 experiments, each assayed in duplicate.)

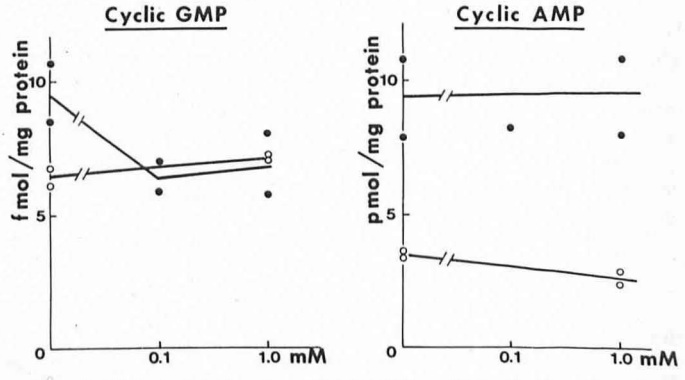

FIG 4. Effect of histamine (H_1) inhibitor, diphenhydramine. After preincubation for 15 min the epidermal squares were incubated for 1 min in Hank's balanced salt solution containing 1 mM histamine and 0, 0.1 and 1.0 mM diphenhydramine (●). Control values, without histamine (○) at 0 and another control, diphenhydramine alone (○) at 1 mM are essentially the same. (An average of 2 experiments, each assayed in duplicate.)

ylate and guanylate cyclase systems (Fig 6). Epinephrine clearly stimulated the adenylate cyclase system, whereas there was no effect on the guanylate cyclase system (note at 5 min of the incubation, the intracellular cyclic AMP level is 1,500-fold higher than the cyclic GMP level). Since this cyclic AMP level did not result in cyclic GMP accumulation in this experiment, we conclude that there is no "spill-over."

The Table summarizes the effects of various hormones and drugs which have been reported to stimulate the guanylate cyclase system in mammalian tissues. None of them convincingly elevated cyclic GMP in epidermis. The baseline cyclic GMP content from skin to skin varies considerably, but within each experiment where control and experimental material are from the same skin the results are reproducible.

DISCUSSION

We have aimed at 2 major questions: (1) what are the hormones or chemicals which can stimulate the epidermal cyclic GMP system?; and (2) does cyclic GMP control epidermal cell mitosis and proliferation?

To answer the first question we have tested the effects of carbachol, concanavalin A, prostaglandin $F_{2\alpha}$, insulin, and vitamin A acid, but none of them convincingly stimulated the epidermal cyclic GMP system. The only drug which stimulated the system was histamine. Since histamine also markedly stimulates the cyclic AMP system [16], we have tried to study its effects on both the cyclic GMP and cyclic AMP systems simul-

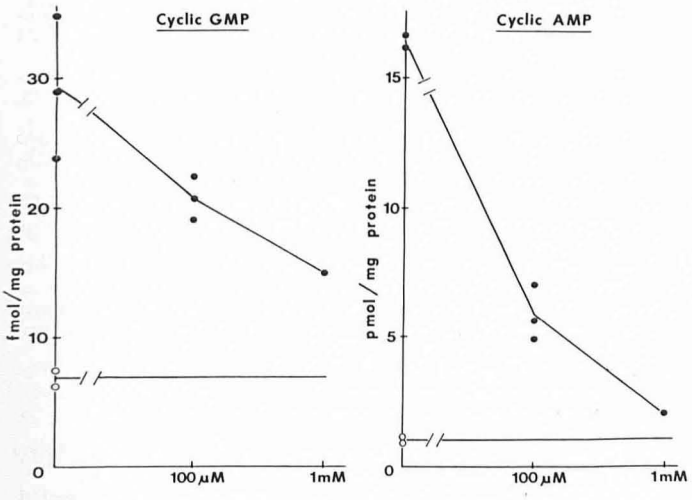

FIG 5. Effect of histamine (H_2) inhibitor, metiamide. Experimental conditions were the same as described in Fig 4 except that the (H_1) inhibitor was replaced by the (H_2) inhibitor at 0, 0.1 and 1.0 mM (●). One of 3 similar experiments is shown. Each point is the average of 2 determinations.

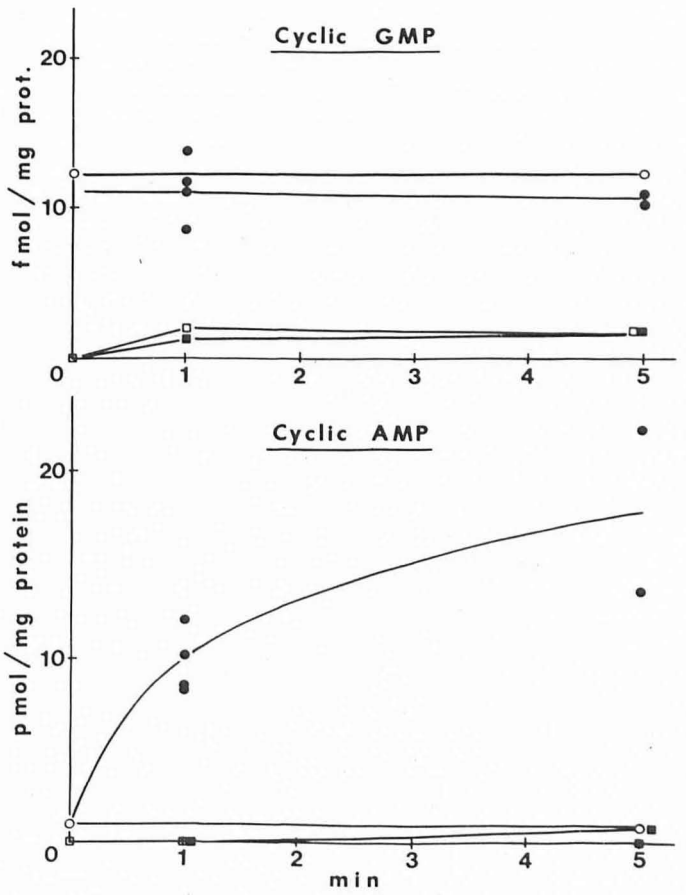

FIG 6. Differential effects of epinephrine on cyclic GMP and cyclic AMP accumulation in pig epidermis. The epidermal squares were incubated in Hank's balanced salt solution with 50 μM epinephrine (●). The control medium (○) contained no epinephrine. No phosphodiesterase inhibitor was added to either medium. Cyclic nucleotide levels in media were measured also (■ = epinephrine, □ = control). One of 3 similar experiments is shown. Each *point* is the average of 2 determinations on 4 combined pieces of skin.

Effects of hormones and chemicals on the accumulation of cyclic GMP (f mol/mg protein ± SE) in the epidermal slices

Hormones & chemicals	Final concl.	Incubation time (min)					
		0	½	1	5	10	20
$PGF_{2\alpha}$	40 μM	18.3 ± 2.0 (n = 12)	—	18.7 ± 3.0 (n = 4)	19.6 ± 2.0 (n = 7)	—	—
Carbachol	1 mM[a]	12.5 ± 1.9 (n = 4)	—	13.0 ± 1.9 (n = 4)	10.0 — (n = 2)	—	—
Insulin	0.4 μg/ml	19.1 ± 1.6 (n = 8)	—	20.3 ± 2.7 (n = 4)	25.2 — (n = 2)	—	—
Vit. A acid	600 μM	30.3 ± 1.4 (n = 4)	32.7	22.6	23.3	37.3	22.0
				(Ave. = 27.6 ± 3.1, n = 2 ea.)			
Con. A	10 μg/ml[b]	18.6 ± 1.9 (n = 6)	20.8	24.1	23.1	14.6	18.7
				(Ave. = 20.3 ± 1.6, n = 2 ea.)			

[a] An additional experiment with the concentration range of 1 μM ~5 mM showed no effect at 1 & 5 min of incubation.
[b] Repeated experiment with 0.2, 2, 20, 200 μg/ml of con A showed also no effect.

taneously and comparatively. The degree of histamine activation of the cyclic GMP system is not extraordinary, but the evidence supporting its positive effect appears to be sufficient. Namely, the time course for cyclic GMP accumulation, and the effects of histamine concentration on the cyclic GMP and cyclic AMP systems are different. Furthermore the histamine-adenylate cyclase system has strictly (H_2) type receptors, whereas the histamine-guanylate cyclase system has both (H_1) and (H_2) but predominantly (H_1) type receptors (Fig 4 and 5).

The occurrence of the histamine-guanylate cyclase system has been described in only 2 organs thus far. One is in guinea pig brain [17] or brain tumor cells [18] and the other in smooth muscle of human umbilical artery [19]. Epidermis is now the third organ reported to have such a system. In the brain system, histamine apparently functions as one of the neurotransmitters, and in the artery histamine increase the level of cyclic GMP which appears to play a role in smooth muscle contraction. In epidermis however, no clear cut function of histamine and cyclic GMP has yet been shown.

In the epidermal outgrowth culture system [11] histamine blocked mitosis at 10^{-6} M, which can be within physiological range. That particular histamine action appeared to be mediated by cyclic AMP, since the addition of dibutyryl cyclic AMP but not dibutyryl cyclic GMP also caused mitotic inhibition [11]. *In vivo* data on the cyclic GMP level in skin consists of a report that cyclic GMP in the lesional epidermis of psoriatics is high as compared with that in the involved epidermis [8]. The steady state level of cyclic AMP in the involved and uninvolved epidermis are about equal [20–22]. At present, the biological role of the histamine-guanylate cyclase system in the epidermis remains unanswered.

REFERENCES

1. Hadden JW, Hadden EM, Haddox MK, Goldberg ND: Guanosine 3′:5′-cyclic monophosphate: A possible intracellular mediator of mitogenic influences in lymphocytes. Proc Natl Acad Sci USA 69:3024–3027, 1972
2. Kram R, Tomkins GM: Pleiotypic control by cyclic AMP: Interaction with cyclic GMP and possible role of microtubules. Proc Natl Acad Sci USA 70:1659–1663, 1973
3. Kimura H, Murad F: Increased particulate and decreased soluble guanylate cyclase activity in regenerating liver, fetal liver, and hepatoma. Proc Natl Acad Sci USA 72:1965–1969, 1975
4. DeRubertis FR, Chayoth R, Field JB: The content and metabolism of cyclic adenosine 3′:5′-monophosphate and cyclic guanosine 3′:5′-monophosphate in adenocarcinoma of the human colon. J Clin Invest 57:641–649, 1976
5. Zeilig CE, Goldberg ND: Cell-cycle-related changes of 3′:5′-cyclic GMP levels in Novikoff hepatoma cells. Proc Natl Acad Sci USA 74:1052–1056, 1977
6. Craven PA, DeRubertis FR: Inhibition by retinol and butylated hydroxyanisole of carcinogen-mediated increases in guanylate cyclase activity and guanosine 3′:5′-monophosphate accumulation. Cancer Res 37:4088–4097, 1977
7. Friedman DL: Role of cyclic nucleotides in cell growth and differentiation. Physiol Rev 56:652–708, 1976
8. Voorhees JJ, Stawiski M, Duell EA, Haddox MK, Goldberg ND: Increased cyclic GMP and decreased cyclic AMP levels in the hyperplastic, abnormally differentiated epidermis of psoriasis. Life Sci 13:639–653, 1973
9. Miller Z, Lovelace E, Gallo M, Pastan I: Cyclic guanosine monophosphate and cellular growth. Science 189:1213–1215, 1975
10. Santoro MG, Philpott GW, Jaffe BM: Inhibition of B-16 meanoma growth in vivo by a synthetic analog of prostaglandin E_2. Cancer Res 37:3774–3779, 1977
11. Flaxman BA, Harper RA: In vitro analysis of the control of keratinocyte proliferation in human epidermis by physiologic and pharmacologic agents. J Invest Dermatol 65:52–59, 1975
12. Iizuka H, Adachi K, Halprin KM, Levine V: Cyclic GMP system in epidermis. I Effect of Ischemia, in press
13. Harper JF, Brooker G: Femtomole Sensitive radioimmunoassay for cyclic AMP and cyclic GMP after 2′0 acetylation by acetic anhydride in aqueous solution. J Cyclic Nucleotide Res 1:207–218, 1975
14. Orr HT, Lowry OH, Cohen AI, Ferrendelli JA: Distribution of 3′:5′-cyclic AMP and 3′:5′-cyclic GMP in rabbit retina in vivo: Selective effects of dark and light adaptation and ischemia. Proc Natl Acad Sci USA 73:4442–4445, 1976
15. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with folin phenol reagent. J Biol Chem, 193:265–275, 1951
16. Iizuka H, Adachi K, Halprin KM, Levine V: Histamine (H_2) receptor-adenylate cyclase system in pig skin (epidermis) Biochim Biophys Acta 437:150–157, 1976
17. Richelson E: Histamine H_1 Receptor-mediated guanosine 3′:5′-monophosphate formation by cultured mouse neuroblastoma cells. Science 201:69–71, 1978
18. Richelson E: Tricyclic antidepressants block histamine H_1 receptors of mouse neuroblastoma cells. Nature 274:176–177, 1978
19. Clyman RI, Sandler JA, Manganiello VC, Vaughan M: Guanosine 3′:5′-monophosphate and adenosine 3′:5′-monophosphate content of human umbilical artery. J Clin Invest 55:1020–1025, 1975
20. Härkönen J, Hopsu-Havu VK, Raji K: Cyclic adenosine monophosphate, adenyl cyclase and cyclic nucleotide phosphodiesterase in psoriatic epidermis. Acta Dermatol Venerol (Stockh) 54: 13–18, 1974
21. Yoshikawa K, Adachi K, Halprin KM, Levine V: Is the cyclic AMP in psoriatic epidermis low? Br J Dermatol 93:253–258, 1975
22. Marcelo CL, Duell EA, Stawiski MA, Anderson TF, Voorhees JJ: Cyclic nucleotide levels in psoriatic and normal keratomed epidermis. J Invest Dermatol 72:20–24, 1979